High Microvascular Blood Volume Is Associated with High Glucose Uptake and Tumor Angiogenesis in Human Gliomas

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INTRODUCTION

Folkman (1) showed in 1971 that the growth, survival, and expansion of a solid tumor is highly dependent on the vascular system recruited by the malignancy. For tumor cell proliferation, invasion, and distant spread, the relationship between vascular neogenesis and tumor cell replication must be synchronous. Previous studies have demonstrated that characterizing the tumor microvasculature on the basis of tissue samples can provide important prognostic information in many malignancies (2), including breast cancer (3, 4), non-small cell lung cancer (5), bladder neoplasias (6, 7), and cervical neoplasia (8, 9). Histological studies of human neoplasms have also demonstrated a correlation between increased vascular density and tumor grade (10, 11).

In parallel with studies of tumor microvasculature, it has been known since the time of Warburg (12) that tumor metabolism may be studied through measurement of glucose consumption. Because glucose is the primary energy source used by normal brain and brain tumors, especially in the hypoxic regime, techniques able to assess the rate at which tumor cells use glucose to fuel their aggressive growth offer a simple method of estimating the overall metabolic activity of the tissue. PET investigations of primary brain tumors using [18F]FDG as a tracer contributed to our knowledge of brain tumor behavior by providing a means of determining regional glucose uptake and consumption (13). PET investigations of glucose metabolism have demonstrated the utility of PET-FDG uptake techniques in evaluating primary brain tumor patients (14, 15). These studies have revealed prognostic information concerning the life expectancy of these patients (16, 17), helped to differentiate tumor

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The abbreviations used are: PET, positron emission tomography; FDG, fluorodeoxyglucose; fMRI, functional magnetic resonance imaging; CBV, cerebral blood volume; TR, repetition time; TE, echo time; ROI, region(s) of interest; CBF, cerebral blood flow; BBB, blood-brain barrier.
recurrence from radiation necrosis (18, 19) despite recent controversy (20), guided the selection of optimal tumor biopsy sites (21, 22), and detected malignant degeneration of low-grade gliomas (23). PET-FDG uptake studies have also indicated that gliomas may contain hotspots of elevated glucose uptake relative to the surrounding brain (13, 24). Of particular interest, these studies demonstrated that FDG uptake is generally higher in high-grade astrocytomas than in low-grade tumors (13), indicating that a greater amount of glucose is consumed in the metabolic processes of high-grade lesions.

Although it is reasonable to hypothesize that the metabolic requirements of brain tumors are mirrored by alterations in cerebral hemodynamics, the regional relationship between tumor vascular proliferation incited by chemical messengers and the regulation of tumor metabolism is poorly understood. Previously, noninvasive in vivo assessment of capillary density with imaging techniques has been impossible because of a lack of appropriate techniques sensitive at the capillary level. fMRI mapping of the relative CBV now provides the spatial and temporal resolution needed to measure regional cerebral blood volume on a voxel-by-voxel basis in both normal (25) and pathological brain tissue (26, 27). Echo planar imaging allows the use of spin-echo pulse sequences with the rapid temporal resolution needed to track the first-pass bolus of contrast agent through multiple slices of the brain (26). These spin echo-based CBV mapping sequences are sensitive primarily to the perfused microvasculature and consequently interrogate these regions specifically (28, 29).

In this work, we measured the potential associations between microvascular proliferation and functional metabolic activity in primary brain tumors. Our primary data consisted of in vivo functional maps of both tumor microvascular CBV (measured using fMRI) and glucose uptake (measured using PET), and ex vivo assessment of tumor microvasculature using quantitative analysis of factor VIII-stained tumor tissue samples. Data were analyzed to address the associations between these parameters both across patients and within each tumor mass. Regions of highest glucose uptake in the PET-FDG uptake studies first were identified and correlated with areas of quantitative analysis of factor VIII-stained tumor tissue samples. Data were analyzed to address the associations between these parameters both across patients and within each tumor mass. Regions of highest glucose uptake in the PET-FDG uptake studies first were identified and correlated with areas of maximum microvascular CBV identified in vivo by functional MRI studies and by factor VIII staining techniques on stereotactic or surgical specimens from the same tumor. fMRI CBV images were then correlated on a voxel-by-voxel basis within each tumor (and contralateral normal brain) with the PET-FDG uptake maps to determine the extent to which areas of functioning vascular density spatially corresponded to regions of increased metabolic activity. Thus, the relationships among glucose uptake, tumor grade, and microvascular blood volume were investigated.

**PATIENTS AND METHODS**

**Patient Selection.** The protocol was approved by the Subcommittee for Human Studies at Massachusetts General Hospital. Glioma patients with histological verification were studied. There were 21 patients, 13 males and 8 females, having a mean age of 44.5 years (range, 25–66 years). Astrocytomas were classified by the scale described by Daumas-Duport et al. (30), and oligodendrogliomas or mixed oligoastrocytomas were classified according to the most malignant component. At the time of imaging, all patients were untreated.

**MRI Protocol.** Conventional MRI images and fMRI CBV maps were acquired during the same procedure from all patients to allow an exact comparison of the results obtained. All MRI studies were performed on a Sigma 1.5 T imager (General Electric, Inc., Milwaukee, WI) retrofitted with Instascan echo planar technology from Advanced NMR, Inc. Sagittal T1-weighted localizer images were acquired. Conventional axial T1- and T2-weighted images then were obtained for each patient. For the dynamic CBV mapping data, a gadolinium-based contrast agent (typically 0.2 mmol/kg) was power-injected (Medrad, Pittsburgh, PA) at an injection rate of 5 ml/s through the angiocatheter.

Dynamic images were acquired from eight slices in 12 subjects, whereas single-slice studies through the center of the tumor mass were acquired for 9 patients with the same imaging parameters. For the multislice studies, the eight slices were collected using a lipid-suppressed spin-echo planar imaging pulse sequence with TR = 1500 ms and TE = 100 ms (64-ms image acquisition window), with 32 images for each slice collected over 48 s before, during, and after each contrast agent injection. For the earlier single-slice studies, a series of 60 images was collected at 1000-ms intervals using the same pulse sequence (TR = 1000 ms; TE = 100 ms). For all studies, a slice thickness of 7 mm was used (full width at half maximum, sinc-shaped radiofrequency slice excitation in fourier space for square slice profile in real space) with an in-plane voxel size of 1.5 × 1.5 mm. After dynamic contrast imaging, a high-resolution, three-dimensional data set was acquired using a T1-weighted, spoiled grass pulse sequence (TR = 40 ms; TE = 5–8 ms; flipangle = 40 degrees; Ref. 31). This data set was used for subsequent image registration to the three-dimensional PET data.

**PET Protocol.** PET was performed parallel to the orbitomeatal line. A molded, plastic face-mask was used to restrict head motion. Images were acquired with either a PC-384 or a PC-4096 PET camera (Scanditronix AB, Uppsala, Sweden). Both instruments are well described in the literature (32, 33). All images were reconstructed using a conventional, filtered back-projection algorithm. An analytic attenuation correction assuming a uniform distribution of absorber within the slice contour (34) was applied to the data. All projection data were corrected for nonuniformity of detector response, dead time, random coincidences, and scattered radiation (35). PET images of glucose uptake were obtained using 18F-labeled FDG. The patients received a 5–10 mCi bolus of [18F]FDG injected i.v. over ~15 s. Imaging was performed 45 min later in two (PC-4096; 30 slices) or three (PC-384; 15 slices) bed positions.

**Immunohistochemistry.** To visualize the microvessels present in the tumor specimens, immunoperoxidase staining for factor VIII, a vascular endothelial component, was performed on 5-μm sections of formalin-fixed, paraffin-embedded biopsied tumor specimens in 17 cases (36). For pathological specimens with multiple blocks, the block chosen for immunohistochemistry was the most representative of the tumor in toto. In a subset of cases, multiple tumor blocks were used to assess issues of tumor heterogeneity. Tumor areas with marked necrosis were not included in the analyses.
After deparaffinization and methanol-H2O2 blocking, non-specific reactions were blocked by incubating the section with normal goat serum (Pierce, Rockford, IL). Tissue sections were then incubated with a polyclonal antibody directed against factor VIII-related antigen (Dako Corp., Carpentry, CA) at a 1:1000 dilution for 16 h at 4°C. After thorough washes with PBS, sections were incubated sequentially with biotinylated goat antirabbit secondary antibody (Vector Labs, Burlingame, CA) and streptavidin-peroxidase complex (Zymed, San Francisco, CA) for 30 min at room temperature for each step, with PBS washes between steps. The antigen-antibody binding was detected with 3,3′-diaminobenzidine-H2O2, and sections were counterstained with hematoxylin, dehydrated, cleared in xylene, and mounted.

Morphometric analysis and quantification of microvascularization were performed using the BioQuant System IV morphometry software package (R&M Biometrics, Nashville, TN) directly from microscope slides containing tumor sections, with the aid of a camera lucida, a hand-held digitizing cursor, and a digitizing tablet. The numbers of individual vessels measured ranged from 38 to 471 for each patient. The number of vessels per unit area (mm2) and vascular area per unit area (mm2) of tumor were computed. The biopsy was assumed to provide a representative sample of the most active area of the tumor tissue for each patient.

Data Analysis. After data collection, CBV maps were derived on a voxel-by-voxel basis from the dynamic image sets (26, 27). Before the starting point of the first-pass circulation (seen as a drop in signal), image data points were averaged and calculated for each voxel as a baseline measure for signal intensity (S0). On a voxel-by-voxel basis, signal was converted to changes in T2 relaxation rate (ΔR2): ΔR2 = −ln(S/S0)/TE

(previous experimental and theoretical data have demonstrated that ΔR2 is approximately linearly related to the contrast agent tissue concentration (37, 38).) Previous experimental and theoretical data have demonstrated that ΔR2 is approximately linearly related to the contrast agent tissue concentration (37, 38).

Relative CBV maps were generated by integration of ΔR2 (contrast agent concentration being linear with blood volume in the well-perfused regime) for each voxel (11, 25, 37). A 3 × 3 voxel smoothing kernel was applied to all raw images prior to integration.

Quantitative comparison was made possible by the acquisition of three-dimensional MRI and PET data sets, which permitted accurate registration of the functional MRI and PET slices within a common coordinate system (40). To make the voxel-by-voxel comparisons on the registered images, ROI for the contralateral, normal hemisphere as well as a tumor region were defined. Because of intrinsic differences in raw spatial resolution of the two imaging modalities, each image was downsampled, using a 3 × 3 mean interpolation kernel, prior to the voxel-by-voxel analysis. This helped to ensure that the intrinsic signal contributing to each observation pair in the calculation of the correlation coefficient originated from an independent spatial region. For each patient, a single, large tumor ROI was then defined on the basis of the T1-weighted conventional image abnormality. The correlation coefficient between the CBV and FDG uptake image data was then calculated for each patient’s tumor ROI. A similar correlation was determined between normal, contralateral gray and white matter across the entire contralateral hemisphere.

For quantitative comparisons across different patients, multiple areas of CBV and FDG uptake were analyzed within each lesion, and the areas of the highest tumor CBV and FDG uptake within each tumor were identified. Because both the PET-FDG uptake technique and the susceptibility contrast CBV mapping method yield “relative” functional information, comparisons among patients were facilitated by reference to an internal standard. As in previous PET work (13), “normal” white matter in the contralateral hemisphere was used for this reference in both fMRI and PET data. White matter ROI were defined on conventional T1-weighted high-resolution magnetic resonance images within normal brain contralateral to the tumor. The maximum CBV and FDG values were normalized to contralateral white matter by dividing them by the mean of this white matter ROI. This first-order normalization accounts for interindividual differences in the hemodynamic and metabolic states of the brain in these patients; the possible systemic effects of the tumor on overall hemodynamic and metabolic state is expected to be small. As an internal control, cortical gray/white matter ratios were also calculated, again using contralateral gray matter ROI defined on high-resolution conventional magnetic resonance images.

Statistical Analyses. Simple linear regression was used to analyze the relationship between maximum tumor CBV measurements and maximum tumor PET-FDG uptake values. Simple linear regression was also used to study the regional CBV and FDG values and their relationships with the number of tumor vessels and their area. For the voxel-by-voxel comparisons, we computed a correlation coefficient of CBV and FDG for all voxels within the tumor ROI as well as for contralateral normal brain area, including both gray and white matter. A two-tailed unpaired t test was used to determine significance between maximum tumor CBV, maximum tumor FDG values, and glioma grade.

RESULTS

Patient information with histological diagnoses is presented in Table 1. Two kinds of analyses were used to quantify the relationship between glucose uptake and microvascular blood volume: a comparison of regions of maximum intensity across patients and voxel-wise analysis within each clinical case. The correlation between the maximum tumor CBV and the maximum tumor FDG uptake, measured by PET, is presented in Fig. 1a. We found a good correlation across all cases (n = 21; correlation coefficient r = 0.572; P = 0.023). The mean maximum tumor CBV in the high-grade glioma group (n = 13) was 4.13 ± 2.92, significantly higher than in the low-grade glioma group (1.51 ± 0.94; n = 8; P = 0.009) with a high-/low-grade ratio of 2.74. The tumor FDG uptake also reflected this difference. The mean maximum tumor FDG uptake in the high-grade glioma group was 2.31 ± 1.03, significantly higher than in the low-grade glioma group (1.29 ± 0.54; P = 0.008) with a high-/low-grade ratio of 1.79. Gray/white matter ratios were measured via both modalities as a measure of reliability, and they compared favorably to previous studies (Ref. 41; CBV: 2.08 ± 0.35; FDG uptake, 2.31 ± 0.68).

The statistically significant correlations of maximum tumor CBV and FDG uptake with the histologically measured number of vessels per unit area of tumor are presented in Fig. 1b (CBV:

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The number density of tumor vessel area and number density tended to be greater in the high-grade glioma group (Table 1). The correlation coefficient $r = 0.827; P < 0.001$; FDG uptake: $r = 0.637; P = 0.006$. As seen in Fig. 1c, the correlation of maximum tumor CBV and tumor vessel area was also statistically significant ($r = 0.757; P = 0.03$). However, the correlation between FDG uptake and measured vascular area was not significant.

Several interesting relationships were found within the morphometric examinations as well. The number density of vessels was found to be correlated with area ($r = 0.752; P < 0.001$) and inversely correlated with average vessel width ($r = -0.618; P < 0.01$), whereas no statistically significant correlation could be found between vessel area and vessel width. Tumor vessel area and number density tended to be greater in the high-grade glioma group ($n = 10$) than in the low-grade glioma group ($n = 7$), but this difference was not statistically significant ($P > 0.05$).

The second method of analysis allowed us to directly compare in vivo the glucose uptake and microvascular blood volume from each tumor in a voxel-by-voxel manner. Fig. 2 shows multislice images obtained from a patient (Table 1, Patient 1) with a left frontal oligoastrocytoma, grade 2/4. The first row (Fig. 2a) is a group of post-contrast agent T1-weighted axial slices; the second row (Fig. 2b) contains the same slices but weighted by T2. Row three (Fig. 2c) contains the derived CBV maps from these slices, and row four (Fig. 2d) contains the corresponding PET-FDG uptake images. Fig. 2e is the voxel-wise plot of PET-FDG uptake versus MRI CBV measurements. In tumor tissue, $r = 0.701$. The difference between the two tissues did not reach significance ($P = 0.36$). Fig. 2f is a sample of the factor VIII staining from which the histological information was determined.

Voxel-by-voxel correlation of MRI CBV results to PET-FDG uptake in normal white and gray matter areas was good in all 21 cases, with correlation coefficients varying between 0.150 and 0.701, with a mean of 0.427. The same correlation between tumor areas on PET-FDG uptake images and CBV maps was found in 16 of 21 patients in the voxel-by-voxel analysis of tumor CBV and FDG uptake; the correlation coefficient varied from 0.0488 to 0.897, with a mean of 0.457. All eight low-grade gliomas exhibited well-correlated voxel-by-voxel glucose uptake and microvascular blood volume. Of the 13 high-grade cases, 8 exhibited well-correlated voxel-by-voxel glucose uptake and microvascular blood volume. Fig. 4 shows a patient (Table 1, Patient 11), with left frontal, temporal, and parietal glioma (astrocytoma, grade 3/4), in which glucose uptake and microvascular blood volume did not correlate.

Table 1  Tumor characteristics, location, histological diagnoses, and correlation indicator

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)/Sex</th>
<th>Tumor location</th>
<th>Histological diagnosis</th>
<th>Voxel-wise correlation</th>
<th>Histology performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>28/F</td>
<td>Left frontal</td>
<td>Oligoastrocytoma, gr. 2/4</td>
<td>+&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>38/M</td>
<td>Left occipital</td>
<td>Astrocytoma, gr. 2/4</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>36/M</td>
<td>Left temporal</td>
<td>Astrocytoma, gr. 2/4</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>38/F</td>
<td>Left temporal</td>
<td>Oligoastrocytoma, gr. 2/4</td>
<td>+&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>25/M</td>
<td>Left frontal</td>
<td>Astrocytoma, gr. 2/4</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>37/M</td>
<td>Left temporal</td>
<td>Astrocytoma, gr. 2/4</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>28/F</td>
<td>Left temporoparietal</td>
<td>Oligoastrocytoma, gr. 2/4</td>
<td>+&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>38/F</td>
<td>Right frontal</td>
<td>Oligoastrocytoma, gr. 2/4</td>
<td>+&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>26/F</td>
<td>Right frontal</td>
<td>Astrocytoma, gr. 3/4</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>48/M</td>
<td>Left frontal</td>
<td>Oligoastrocytoma, gr. 3/4</td>
<td>+&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>50/M</td>
<td>Left frontal, temporal, parietal</td>
<td>Astrocytoma, gr. 3/4</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>50/M</td>
<td>Right temporal</td>
<td>Oligoastrocytoma, gr. 4/4</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>13</td>
<td>62/M</td>
<td>Left temporal</td>
<td>Astrocytoma, gr. 3/4</td>
<td>+&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>66/M</td>
<td>Left parietal</td>
<td>Astrocytoma, gr. 4/4</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>48/F</td>
<td>Right frontal</td>
<td>Astrocytoma, gr. 3/4</td>
<td>+&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>63/M</td>
<td>Right frontal</td>
<td>Oligoastrocytoma, gr. 3/4</td>
<td>+&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>60/F</td>
<td>Right temporal</td>
<td>Astrocytoma, gr. 4/4</td>
<td>+&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>43/M</td>
<td>Right frontal</td>
<td>Astrocytoma, gr. 3/4</td>
<td>+&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>45/F</td>
<td>Left parieto-occipital</td>
<td>Astrocytoma, gr. 4/4</td>
<td>+&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>41/M</td>
<td>Right frontal</td>
<td>Astrocytoma, gr. 4/4</td>
<td>+&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>62/M</td>
<td>Left frontal</td>
<td>Astrocytoma, gr. 3/4</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

<sup>a</sup> gr., grade.<br><sup>b</sup> +, positive correlation; –, no correlation.<br><sup>c</sup> +, histology performed; –, histology not performed.

DISCUSSION

Our investigation indicates that there is an association between microvascular density and tumor energy metabolism in normal brain and most of the human gliomas. This study confirms that susceptibility contrast fMRI CBV mapping is sensitive for perfused microvascular structures (functioning capillaries and microvessels; Refs. 28, 42). Good correlation was also
observed between regions of maximum CBV, determined by fMRI and verified by immunohistochemical analyses, and the regions of maximum glucose uptake by tumor cells, as measured by PET-FDG uptake. Finally, a statistically significant correlation was derived between a microvascular area assumed to adequately sample vascular density, determined from immunohistochemical morphometric studies, and maximum glucose uptake, evaluated by PET-FDG uptake in vivo.

The correlation between blood volume and glucose uptake in the normal brain is not unexpected: glucose is the most readily available energy source for normal brain cells. In normal brain tissue, physiological changes in the regional CBV provide an efficient means of regulating regional CBF. This relationship between CBV and CBF has been observed in PET studies (43, 44) and held for regions of maximum intensity in both modalities as well as voxel-by-voxel comparison over tumor tissue and normal tissue. The gray/white matter ratios measured are as expected and lend further credence to the reliability of the FDG and CBV measurements (27, 41).

Having demonstrated the link between regions of maximum blood volume and glucose uptake, and the efficacy of our methods for imaging this relationship, we then focused on the voxel-by-voxel analysis. Our data from the voxel-by-voxel image comparisons across patients indicated that CBV at the voxel level and glucose uptake are strongly associated in most of the gliomas, even regionally. Contrary to the situation in healthy brain, the regulatory function of the microvascular bed may not be flow dependent in tumor tissue, where the microvascular density and the capillary surface area may provide an upper limit for the transport mechanism between the capillary bed and extravascular structures.

In the low-grade gliomas, a typical finding was that the tumor area was characterized by low CBV and corresponded well with low glucose uptake in the PET-FDG uptake images (Fig. 2). High-grade tumors demonstrated regions characterized by both high CBV and high glucose uptake (Fig. 3). In the case of the high-grade gliomas, however, both PET-FDG uptake and MRI CBV mapping studies often demonstrated considerable heterogeneity.

A previous PET study demonstrated that tumor CBV increased both in absolute value and relative to the values obtained in the contralateral cortex (45). This does not contradict our results. Our basic assumption is that PET blood volume mapping (based on PET CO techniques, which are sensitive for erythrocytes in the blood) and MRI spin-echo blood volume mapping (sensitive to the microvasculature plasma volume) measure different structures and their related functions. When the BBB is intact, the MRI CBV map reflects the regional microvascular cerebral blood volume correctly, although when grossly disrupted, the MRI CBV map may underestimate the

Fig. 1  

| a | Correlation between dimensionless maximum tumor FDG and maximum tumor CBV (n = 21 patients; r = 0.572). | b | Plot of maximum tumor CBV (●) and FDG (○) versus number of tumor vessels per unit area (n = 17 patients; CBV: r = 0.827, P < 0.001; FDG: r = 0.637, P = 0.006). | c | Plot of maximum tumor CBV (●) and FDG (○) versus tumor vessel area (n = 17 patients; CBV: r = 0.757, P = 0.03; FDG: P > 0.1). |
Fig. 2 Images from patient 1 with a left frontal oligoastrocytoma, grade 2/4 containing relatively low, homogeneous tumor CBV. 

- **a**, post-contrast injection T1-weighted images, nonenhancing; 
- **b**, T2-weighted images; 
- **c**, CBV maps; 
- **d**, PET-FDG glucose uptake; 
- **e**, voxel-by-voxel correlation between PET-FDG uptake and MRI CBV measurements (rCBV) in the homogeneous low-grade tumor (■; \( r = 0.841 \)) and in normal tissue (×; \( r = 0.701 \)); 
- **f**, factor VIII staining confirming low number of tumor vessels in this patient. This case demonstrates a typical finding of a low-grade glioma where low microvascular CBV is associated with low FDG uptake.
actual regional blood volume (26), depending on the degree of BBB disruption. For untreated intra-axial tumor, however, the degree of BBB disruption is typically almost an order of magnitude smaller than the vascular permeability observed in other tumors such as extra-axial lesions; thus, BBB permeability effects are unlikely to contribute significantly to errors in this study.

A striking feature of astrocytoma tumor progression is an increase in neovascularization (46). The expression of angiogenic genes together with receptors on the tumor cells and endothelial cells of primary human gliomas may support endothelial proliferation and contribute to neoplastic growth and progression (47). Although the exact mechanism of angiogenesis regulation in human gliomas is incompletely characterized, the tumor’s energy (affected by CBF) and oxygen metabolism (affected by CBV) may play a key role (48). Therefore, by imaging glucose uptake and microvascular blood volume, we are in effect imaging two important aspects of tumor angiogenesis: microvascular blood volume, accentuating sprouts and capillaries, and metabolic glucose energy utilization.

Another perspective on the relationship between neovascularity and tumor metabolism comes from inspection of the regional regression relationship between these two parameters, as provided by our coregistered image data sets. The regression of our plots of FDG versus CBV can be interpreted as a measure of the total microvascular volume supporting a given tissue glucose utilization. In many cases, both the tumors and normal brain tissue had essentially the same slopes and intercepts (see Fig. 2c and Fig. 3e). However, there was considerable variation in the points having high FDG and CBV values. This somewhat surprising finding suggests that the fundamental relationship between tissue microvascular density and glucose utilization remained constant between tumors and normal brain, despite the dramatic histological and biological differences between these tissues. In some cases, however, tumor voxels were observed to lie dramatically below the observed regression for normal tissue; in other words, these lesions showed regions of decreased glucose utilization per unit microvascular volume (e.g., Fig. 4). Of such cases, high-grade lesions showed the smallest slopes (lowest glucose utilization per unit microvascular blood volume).

Together, these findings suggest that although glucose utilization and microvascular proliferation are often closely linked, they must ultimately be controlled by distinct underlying...
Fig. 3 Images from patient 10 with a left frontal oligoastrocytoma, grade 3/4 containing relatively high tumor CBV. a, post-contrast injection T1-weighted images, enhancing; b, T2-weighted images; c, CBV maps; d, PET-FDG glucose uptake; e, voxel-by-voxel correlation between PET-FDG uptake and MRI CBV measurements ($r_{CBV}$) in the homogeneous high-grade tumor (●, $r = 0.646$) and in normal tissue (×, $r = 0.257$); f, factor VIII staining demonstrates an increased number of vessels in the tumor tissue in this patient. This case demonstrates a typical finding of a high-grade glioma in our series.
genomic factors. The observation of high-grade individual tumor regions with low glucose utilization and high microvascular volume does not exclude the possibility that during malignant dedifferentiation, microvascular proliferation is a necessary step in supporting increased metabolic activity and may foreshadow it, but may not itself be directly responsible for that increase. These data suggest the hypothesis that the process of malignant dedifferentiation of these lesions occurs as a series of independent genetic mutations (49) and that those responsible for encouraging tumor vascular proliferation occur before tumors can support increased metabolic activity. Longitudinal studies of patients presenting with low-grade malignancies would be informative in determining whether changes in tumor angiogenesis and metabolism are temporally displaced, and on average, by how long. Such studies have important clinical implications in the functional imaging of glioma tumor dedifferentiation and progression with respect to choosing the optimal imaging modality for detection of malignant degenerative changes and on the frequency with which such studies should be performed.

On the basis of the findings in the present work, as well as the results of previous studies, a link is postulated between tumor metabolism, tumor angiogenesis, and microvascular density (10, 46). The regulation of tumor angiogenesis is complex and involves a close interaction between tumor cells, the surrounding extracellular matrix, and endothelial cells. Regulation also depends on various pathophysiological parameters, including interstitial pressure, intracellular pH, and various growth factors (50). Although neovascularization of the malignant tumor may be a necessary prerequisite for the continued growth of the lesion, as documented earlier, there still exists the possibility that increased tumor metabolism may itself induce angiogenesis through various mechanisms. Alternatively, increased energy consumption and active angiogenesis may also reflect a common underlying mechanism of deregulation.

The data presented herein establish a link between tumor cell metabolic activity and functioning microvascular density. Our data show a correlation between tumor microvascular blood volume, measured both in vivo by fMRI and in excised speci-
mens, and tumor glucose uptake. These findings suggest that promotion of active tumor angiogenesis in adult human gliomas is linked to increased tumor metabolic activity and malignant potential both across tumors and regionally within a single, heterogeneous tumor. This discovery could have important clinical applications in imaging the natural history of glial tumor initiation and progression, especially in the longitudinal monitoring of glioma patients.

REFERENCES

Fig. 4 Images from patient 11, with a large left frontal, temporal, and parietal glioma, grade 3/4, in which CBV and FDG did not correlate well ($r = 0.0488$). a, post-contrast injection T1-weighted image, nonenhancing; b, MRI CBV image; c, PET-FDG glucose uptake; d, voxel-by-voxel comparison of PET-FDG uptake and MRI CBV measurements ($r_{CBV}$). The patient exhibited predominantly low CBV with a focal area of increased CBV that did not correspond to increased glucose uptake.


High Microvascular Blood Volume Is Associated with High Glucose Uptake and Tumor Angiogenesis in Human Gliomas

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